

QUALITY ASSESSMENT OF COLD-PRESSED STRAWBERRY, RASPBERRY AND BLACKBERRY SEED OILS INTENDED FOR COSMETIC PURPOSES

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ABSTRACT

Background. Cold-pressed berry seed oils are used for consumption and other applications including skin and hair care. They are natural products which gain the attention of customers. In this study, strawberry, raspberry and blackberry seed oils used for cosmetic purposes, purchased from three different European producers, were analyzed. The aim of the study was to assess the quality and oxidative stability of the berry fruit oils, thus they were analyzed after purchase and after 4 and 8 weeks of storage at room temperature.

Materials and methods. Acid and peroxide values were determined in the tested oils, as was oxidative stability, which was measured using pressure differential scanning calorimetry (PDSC). Additionally, fatty acid profiles and their distribution at sn-1,3 and sn-2 positions of triacylglycerols were characterized.

Results, principal. Most of the fatty acids of the tested berry seed oils are polyunsaturated fatty acids (67.04–74.95%). The results show the low quality of the tested oils in terms of oxidative stability (high peroxide values: 21.9–249.6 mEq O₂/kg oil).

Conclusion. Based on this study, it is necessary to evaluate the effects of these products on the body. Moreover, standards clarifying the oxidation of cosmetic oils should be set internationally.

Keywords: raspberry oil, strawberry oil, blackberry oil, oxidative stability, fatty acids, PDSC

ABBREVIATIONS

PDSC – pressure differential scanning calorimetry
SFA – saturated fatty acids
MUFA – monounsaturated fatty acids
PUFA – polyunsaturated fatty acids
OTI – oxidation induction time
TAG – triacylglycerol
PV – peroxide value
AV – acid value
AOCS – American Oil Chemists' Society

INTRODUCTION

In the European classification, strawberries are low growing berries, while raspberries and blackberries are cane berries. Strawberry seeds represent 1% of the weight of the fruit, but around 20% of the seeds is oil (Grzelak-Błaszczak et al., 2017). Approximately 10% of fresh raspberries are seeds with around 23% of that being oil (Johansson et al., 1997), while for fresh blackberries, accordingly app. 5% are seeds with an oil content of around 14% (Dimić et al., 2012). Fruit

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seeds are major by-products of the food industry, so finding new applications would promote their sustainable production.

Berry seed oils, including strawberries, raspberries and blackberries, are widely used in cosmetics production. These oils are often added to creams, shampoos, bath oils, lipsticks and many other cosmetics to give a berry aroma and to provide essential nutrients. PUFA are claimed to be essential for normal cell structure (Johansson et al., 1997; Mildner-Szkudlarz et al., 2019).

Over the last decades, consumers have expressed more interest in natural products, not only in the context of food but also in cosmetics and medicines. This interest has challenged scientists and producers to search for and use raw materials and sources. The use of fruit seeds, which are usually by-products in the food industry, is one of the latest trends (Glampedaki and Dutschk, 2014).

Cold-pressed oils are important products on the food market, but for years they have also been in cosmetics, applied to skin, hair and nail care (Ligeza et al., 2016). Products which come into contact with human skin must be non-hazardous to health. Cold-pressed oils are obtained by mechanical treatment, e.g., pressing, without heating. The temperature of the pressed oil has to be kept below 40°C. It can be purified by filtering, settling or centrifuging only.

Consumer interest in so-called ecological and natural products has increased recently. There are several reasons for this, such as the growing number of reports on the harmful effects of chemical substances widely used in cosmetics production. Secondly, the negative impact of cosmetics production on the environment. Berry seed oils are becoming more popular due to their health benefits, linked to their high content of PUFA and antioxidants.

Polyunsaturated fatty acid oxidation is the main reaction that affects the quality of oil during processing, storage and use (Micić et al., 2015). Biologically active substances such as carotenoids, tocopherols and chlorophylls present in unrefined oils influence the stability of the oils and provide them with an attractive colour (Harhaun et al., 2020).

Studies evaluating oils intended for cosmetic purposes obtained from berry seeds are lacking, therefore, this research was carried out to assess the quality of these products.

MATERIALS AND METHODS

Chemicals

All of the reagents and solvents used, of the HPLC/GC purity, were obtained from POCH S.A. (Gliwice, Poland). The Supelco 37 Component Fatty Acid Methyl Esters (FAME) mix standard was supplied by Sigma-Aldrich GmbH (Schnelldorf, Germany).

Materials

The tested oils were purchased in retail outlets in Europe in May 2019 in brown, glass bottles of 50 mL volume. Three strawberry (*Fragaria*) (S1, S2, S3), three raspberry (*Rubus idaeus*) (R1, R2, R3) and three blackberry (*Rubus fruticosus*) (B1, B2, B3) cold-pressed oils were analyzed. The oils were sold as cosmetics recommended for dry skin. Producers advertise that regular skin treatment should effectively moisturize, regenerate and protect the skin.

Acid value (AV) and peroxide value (PV)

Hydrolytic changes in the tested oils were specified by the acid values according to the AOCS method (AOCS Official Method Te 1a-64). Peroxide content and the primary oxidation of the oils were tested based on peroxide values according to the AOCS method (AOCS Official Method Cd 8b-90). The acid and peroxide values were measured after purchase and after storage at room temperature (around 20°C) in the original bottles over periods of 4 and 8 weeks.

Fatty acid composition

Determination of esterified fatty acids in the tested oils was done according to the AOCS method (AOCS Official Method Ce 1h-05). Preparation of fatty acid methyl esters was done according to the AOCS method (AOCS Official Method Ce 2-66). A Young Lin instrument model YL6100 gas chromatograph (Young Lin Instrument Co., Ltd, Korea) with a flame ionization detector equipped with a BPX-70 column (60 m × 0.25 mm × 0.25 µm) was used. The injector temperature was set to 225°C. The injection was maintained in the split mode (1:25). The operating conditions were as follows: nitrogen flow 1 mL/min, oven temperatures: initial temperature 70°C/0.5 min, before increasing to 160°C at a rate of 15°C/min, followed by a temperature increase from 160°C to 200°C at a rate of 1.1°C/min;

200°C/12 min, temperature rise from 200°C to 225°C at a rate of 30°C/1 min; a final temperature of 225°C/1 min. Detector temperature was set at 250°C. Peaks were identified by comparing retention times with the Supelco 37 Component FAME Mix standard.

The positional distribution of fatty acids of triacylglycerols (sn-2 and sn-1,3)

Enzymatic hydrolysis of TAGs was carried out by applying pancreatic lipase specific for ester bonds taking the sn-1,3 position. Hydrolysis was done in a laboratory shaker for 20 min at 40°C. 8 mL of an aqueous solution of TRIS at pH 8.0 and a concentration of 1 mol/dm³, as well as 0.5 mL of a 2.2% CaCl₂ solution and 0.2 cm³ of a 1% aqueous solution of bile salts was added to a flask containing 400 mg of oil. For 10 min the contents of the flask were kept at 40°C, then 200 mg of pancreatic lipase was added. The reaction was stopped by adding 15 mL of ethyl alcohol and 5 mL of 6 mol/dm³ hydrochloric acid. The products obtained during hydrolysis were extracted three times in a separatory funnel with diethyl ether. The organic phase was washed three times with distilled water until the indicator paper became neutral and then dried with anhydrous magnesium sulfate. Enzymatic hydrolysis products dissolved in diethyl ether were separated by preparative thin-layer chromatography (TLC). For this purpose, 20×20 cm silica gel plates were used. A chromatograph was developed using a mixture of 50 mL of hexane, 50 mL of diethyl ether and 1 mL of acetic acid. Silica gel, along with isolated sn-2 monoacylglycerols, was removed from the plates. The fat substance was eluted with diethyl ether (Luddy et al., 1964). The percentage of selected fatty acids at the sn-2 positions of monoacylglycerols was also calculated in relation to the total content of a given acid at all positions of triacylglycerols.

Pressure differential scanning calorimetry

Oxidative stability was determined using a pressure differential scanning calorimeter (model Q20, TA Instruments). The instrument was calibrated using standard, high purity indium. Oil samples of 3.0–4.0 mg were transferred to aluminum vessels, then placed in the calorimeter pressure sample chamber, and then oxidized isothermal (120°C) at a constant oxygen pressure between 1380 and 1400 kPa. The result of each

measurement was a diagram representing a recorded heat flux flowing through the sample in time, measured against a reference system. The diagrams were analyzed using TA Universal Analysis 2000 software. The oxidation induction time (OTI) was determined based on the maximum rate of oxidation. The PDSC test was done oils after purchase and after storage at room temperature in the original bottles over periods of 4 and 8 weeks.

Calculations and statistics

The statistical program Statistica 13.3 was used to develop the results. The results were developed using a two-factor analysis of variance and a Tukey test at a significance level of $\alpha = 0.05$, by which homogeneous groups were determined. The mean of the results and standard deviations were calculated in Microsoft Excel. The determinations were carried out in triplicate.

RESULTS AND DISCUSSION

Most of the results in the literature are about berry seeds oils for edible applications. In contrast, the oils evaluated in this study are for nonedible application, thus some of the results may strongly differ in comparison with published data.

Acid and peroxide values

All the tested oils had low acid values (Table 1) at the beginning (2.05–3.45 mg KOH/g oil) and after 8 weeks of storage (2.75–4.58 mg KOH/g oil), which proves the low degree of hydrolysis and the small amount of free fatty acids. Acid values in other studies were significantly higher, for example, the blackberry and raspberry oils reported by Dimić et al. (2012) were respectively 6.85–7.05 and 17.18–17.86 mg KOH/g oil. Another study (Mildner-Szkudlarz et al., 2019) presents similar to the one obtained in this study results for cold-pressed strawberry seed oil (3.55 mg KOH/g) and raspberry oil (4.12 mg KOH/g).

The peroxide values of the cosmetic oils in this study were relatively high at the beginning of the test, with strawberry oils (57.2–89.2 mEq O₂/kg oil), raspberry oils (21.9–58.9 mEq O₂/kg oil) and blackberry oils (82.4–249.6 mEq O₂/kg oil) all above the values reported in the literature for edible oils (Dimić et al.,

Table 1. Changes of chemical parameters in oils after storage

Sample	Storage weeks	Acid value mg KOH/g oil	Peroxide value meq O ₂ /kg oil	OTI at 120°C min
S1	0	2.71 ±0.08	75.6 ±1.4	12.8 ±0.3
	4	2.97 ±0.15	88.0 ±4.1	10.7 ±0.3
	8	3.25 ±0.07	121.2 ±0.9	9.0 ±0.1
S2	0	2.05 ±0.07	57.2 ±2.6	19.5 ±1.2
	4	2.49 ±0.05	78.8 ±4.4	17.4 ±0.3
	8	2.75 ±0.15	86.8 ±0.3	15.1 ±0.3
S3	0	3.02 ±0.01	89.2 ±1.3	15.9 ±0.4
	4	4.36 ±0.16	95.9 ±1.2	13.5 ±0.5
	8	4.58 ±0.01	115.5 ±1.1	12.8 ±0.4
R1	0	2.60 ±0.21	24.1 ±0.3	47.6 ±1.9
	4	2.93 ±0.05	53.3 ±0.9	45.8 ±0.7
	8	3.18 ±0.22	91.6 ±2.7	40.4 ±0.5
R2	0	3.44 ±0.34	21.9 ±5.3	49.4 ±2.4
	4	3.48 ±0.11	39.6 ±2.4	46.5 ±0.2
	8	3.86 ±0.07	69.1 ±0.7	41.6 ±0.4
R3	0	3.45 ±0.12	58.9 ±1.1	38.4 ±1.1
	4	3.68 ±0.01	85.4 ±0.5	33.2 ±0.6
	8	4.14 ±0.22	118.5 ±3.2	27.8 ±0.9
B1	0	2.72 ^a ±0.07	126.5 ±1.5	25.7 ±0.7
	4	3.00 ±0.35	159.8 ±4.4	23.3 ±1.2
	8	3.47 ±0.07	187.7 ±1.1	21.5 ±0.4
B2	0	2.18 ^a ±0.08	82.4 ±0.3	38.4 ±1.9
	4	2.29 ^a ±0.10	102.9 ±1.4	35.9 ±0.5
	8	3.39 ±0.07	129.3 ±11.3	31.4 ±0.3
B3	0	3.13 ±0.17	249.6 ±0.4	12.5 ±5.2
	4	3.29 ±0.01	282.5 ±0.1	12.2 ±0.7
	8	3.73 ±0.07	320.5 ±0.2	6.8 ±0.4

S1, S2, S3 – strawberry seed oils; R1, R2, R3 – raspberry seed oils; B1, B2, B3 – blackberry seed oils.

Presented data are means ±SD (*n* = 6). OTI – oxidation induction time.

2012; Mildner-Szkudlarz et al., 2019; Oomah et al., 2000; Parry and Yu, 2004). More oxidation products (Table 1) were formed after 4 and 8 weeks of storage of the analyzed oils, and the PVs increased significantly, between 86.8–121.2 (strawberries), 69.1–118.5 (raspberries) and 129.3–320.5 mEq O₂/kg (blackberries).

Based on the other studies of cold-pressed berry oils, the peroxide values of raspberry seed oils were between 4.4–46.5 mEq O₂/kg, of blackberry seed oil 30.3–91.0 mEq O₂/kg and of strawberry oils 29.1–30.3 mEq O₂/kg (Oomah et al., 2000; Parry and Yu, 2004). The results reported by other authors also show high peroxide values, which are far above the standard for edible cold-pressed oils (Codex Alimentarius, 2009). The results indicate an exceeded level of primary oxidation in the tested oils.

Fatty acids composition and distribution

All the tested berry seed oils (Table 1) contained a small percentage of SFA (5.87–8.62%) and a high level of PUFA (67.04–74.95%). The composition of strawberry seed oils differed depending on the producer. Approximately half of the fatty acids (38.68–51.03%) was linoleic acids (LA). The strawberry seed oils had a high content of alpha-linolenic acid – ALA (15.95–34.89%) and a significant amount of oleic acid (16.24–22.54%). These results correspond with the results of other authors: 48.5% LA, 30.5% ALA, and 14.5% oleic acid (Dubois et al., 2007; Mildner-Szkudlarz et al., 2019). The fatty acid profile of raspberry seed oils is characterized by a high amount of linoleic acid (52.81–55.75%), then oleic acid (18.50–20.35%) and alpha-linolenic acid (15.70–20.94%). Other studies show that the content of LA was also around 55%, but ALA was a bit higher (27.80–33.67%) and oleic acid lower (11.26–12.11%) in raspberry seed oil (Micić et al., 2015; Mildner-Szkudlarz et al., 2019; Oomah et al., 2000). In the composition of blackberry seed oils, LA has the highest percentage (59.90–61.34%), followed by oleic acid (19.47–20.94%), and alpha-linolenic acid – ALA (8.25–8.72%). A slightly different fatty acid composition was reported in blackberry seed oil by other authors (Micić et al., 2015): LA (66.33%), ALA (14.62%), oleic acid (12.53%).

The fatty acids are selectively esterified to the glycerol skeleton at three stereo-specifically numbered

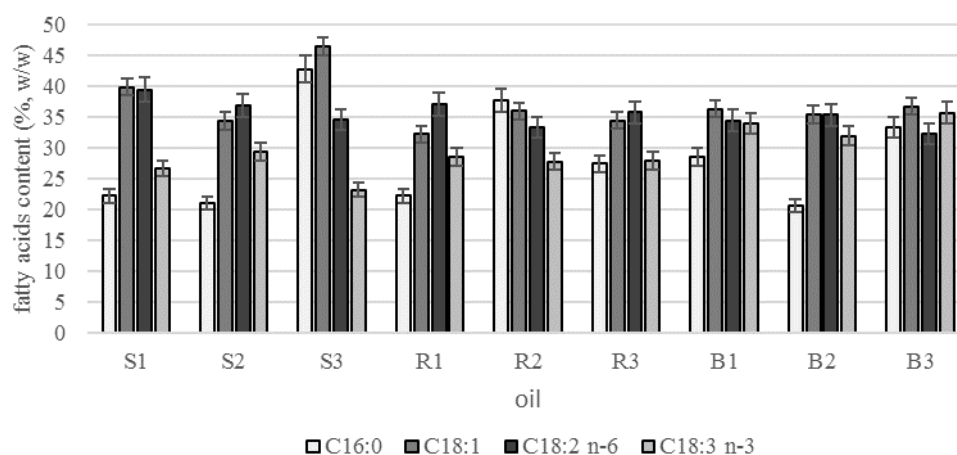


Fig. 1. Percentage of fatty acids at sn-2 position in triacylglycerols in the oils, % w/w: S1, S2, S3 – strawberry seed oils; R1, R2, R3 – raspberry seed oils; B1, B2, B3 – blackberry seed oils

(sn) positions. The proportion of sn-2 to sn-1,3 can significantly affect the uptake, metabolism and tissue distribution of fatty acids (Dubois et al., 2007). Fatty acid distribution in TAGs differs depending on the berry and fatty acid. It was observed that oleic and alpha-linolenic acids occurred mainly in the internal position of the TAGs of all berry seed oils (Fig. 1). The percentage of ALA at sn-2 of triacylglycerols of strawberry seed oils does not exceed 33%, which means that it is mostly in the external positions of TAGs. The distribution of alpha-linolenic acid between the external and internal positions of triacylglycerols in raspberry and blackberry seed oils tends to be balanced. In most of the tested oils, saturated palmitic acid occurred at the sn-2 position of the TAGs (Table 3).

Oxidative stability

DSC and PDSC are applied in the measurement of the oxidative stability of different types of oils (Micić et al., 2015). Oxidative stability is considered to be an important factor affecting the quality and safety of cosmetics. Based on the thermo-analytical method, the shortest oxidation induction time was seen for strawberry seed oils, followed by raspberry and blackberry oils (Table 1). The more unsaturated fatty acids (mainly ALA), the shorter the OTI (Table 2). Compared to other studies of seeds oils, OTI is relatively short and is also affected by the primary oxidation (Raczyk et al., 2016). Oils with high PVs had a short induction time after 8 weeks of storage (Table 1). Such short OTIs were found for linseed (19.8–25.3 min) and camelina (27.9–32.2 min) oils measured by PDSC at 100°C (Raczyk et al., 2016). A longer time was found

Table 2. Fatty acids composition in the oils, %

Fatty acids % ±SD	Strawberry oil			Raspberry oil			Blackberry oil		
	S1	S2	S3	R1	R2	R3	B1	B2	B3
1	2	3	4	5	6	7	8	9	10
C16:0	4.60 ±0.02	5.46 ±0.08	4.36 ±0.09	4.79 ±0.10	4.32 ±0.05	3.99 ±0.04	4.84 ±0.04	5.42 ±0.04	5.07 ±0.04
C 18:0	1.94 ±0.09	2.61 ±0.10	1.92 ±0.04	2.15 ±0.03	2.55 ±0.06	1.88 ±0.02	3.42 ±0.08	3.20 ±0.03	3.48 ±0.07

Table 2 – cont.

1	2	3	4	5	6	7	8	9	10
C18:1	17.29 ±0.04	22.54 ±0.05	16.24 ±0.03	20.35 ±0.12	19.74 ±0.05	18.50 ±0.01	19.47 ±0.06	20.94 ±0.05	20.31 ±0.03
C18:2 n-6	38.69 ±0.11	51.03 ±0.05	46.24 ±0.06	53.64 ±0.14	55.75 ±0.15	52.81 ±0.04	61.34 ±0.09	59.90 ±0.01	59.92 ±0.12
C18:3 n-6	0.13 ±0.01	0.06 ±0.02	0.11 ±0.01	0.07 ±0.03	0.06 ±0.02	0.09 ±0.03	0.04 ±0.01	0.04 ±0.01	0.04 ±0.02
C18:3 n-3	34.89 ±0.08	15.95 ±0.06	28.60 ±0.08	17.18 ±0.04	15.70 ±0.07	20.94 ±0.02	8.57 ±0.08	8.25 ±0.04	8.72 ±0.09
Other	2.46	2.35	2.53	1.82	1.88	1.79	2.32	2.25	2.46
∑ SFA*	6.54	8.07	6.28	6.94	6.87	5.87	8.26	8.62	8.55
∑ MUFA*	17.29	22.54	16.24	20.35	19.74	18.50	19.47	20.94	20.31
∑ PUFA*	73.71	67.04	74.95	70.89	71.51	73.84	69.95	68.19	68.68

Each value represents the mean of three replicates ±standard deviation.

*Calculated values.

Table 3. Fatty acids composition in the oils in sn-2 and sn-1,3 positions of triacylglycerols, %

Fatty acids, %	Strawberry oils			Raspberry oil			Blackberry oil		
	S1	S2	S3	R1	R2	R3	B1	B2	B3
C16:0									
sn-2	2.95	3.35	5.49	3.47	4.85	3.26	4.09	3.17	5.08
sn-1,3	5.16	6.25	3.68	6.08	4.01	4.30	5.13	6.12	5.08
C18:1									
sn-2	20.75	23.31	22.71	20.17	21.47	19.16	21.21	22.25	22.46
sn-1,3	15.66	22.25	13.09	21.21	19.09	18.21	18.60	20.27	19.36
C18:2									
sn-2	45.79	56.39	47.97	58.97	55.71	56.59	63.246	63.79	57.86
sn-1,3	35.22	48.36	45.43	50.05	55.65	50.76	0.24	58.26	60.66
C18:3									
sn-2	27.90	14.05	19.82	14.38	12.97	17.55	8.75	7.89	9.36
sn-1,3	38.33	16.85	32.77	18.01	16.87	22.61	8.51	8.40	8.43

using the DSC method, with OTI for blackberry seed oil at 120°C being 138.3 min, and for raspberry seed oil 72.3 min (Micić et al., 2015). However, these results were obtained without applying high pressure to the samples.

CONCLUSIONS

Cold-pressed berry seed oils could be of excellent use in fruit industry by-products. Strawberry, raspberry and blackberry seed oils can be a valuable ingredient in skin and hair care products. However, it is important to assess their safety and quality. Berry seed oils can be attractive because of their polyunsaturated fatty acids and bioactive compounds. However, the research shows a low oxidative stability for the samples tested. The high content of oxidative products has a negative effect on the quality of the oils, so this needs to be kept under control.

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